EuFishBioMed (COST Action BM0804): A European network to promote the use of small fishes in biomedical research.

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Abstract

Small fresh water fishes such as the zebrafish (*Danio rerio*) have become important model organisms for biomedical research. They currently represent the best vertebrate embryo models in which it is possible to derive quantitative data on gene expression, signalling events and cell behavior in real time in the living animal. Relevant phenotypes in fish mutants are similar to those of other vertebrate models and human diseases. They can be analysed in great details and much faster than in mammals. In recent years, approximately 2,500 genetically distinct fish lines have been generated by European research groups alone. Their potential, including their possible use by industry, is far from being exploited.

To promote zebrafish research in Europe, EuFishBioMed was founded and won support by the EU COST programme (http://www.cost.esf.org/). The main objective of EuFishBioMed is to establish a platform of knowledge exchange for research on small fish models with a strong focus on widening its biomedical applications and an integration of European research efforts and resources. EuFishBioMed currently lists more than 300 member laboratories in Europe, offers funding for short-term laboratpry visits, organizes and co-sponsors meetings and workshops and has successfully lobbied for the establishment of a European Zebrafish Resource Centre. To maintain this network in the future, beyond the funding period of the COST Action, we are currently establishing the European Society for Fish Models in Biology and Medicine.

Background

Small freshwater fishes such as zebrafish (*Danio rerio*) are increasingly being used as model organisms for biomedical research offering sequenced genomes and a rich repertoire of genetic, molecular and cellular manipulation tools[1, 2]. A unique set of properties (small size, numerous offspring, optical transparency of the embryo, amenability to genetic and chemical screens) has made them popular vertebrate animal models[2, 3]. It has become clear that the potential of small fish models far exceeds their traditional usage in the fields of cell biology and developmental genetics. Zebrafish models have been described for a large number of human conditions including, but not limited to, polycystic kidney disease[4, 5], heart arrhythmias[6], congenital heart defects and cardiomyopathies[7] and myopathies of the skeletal musculature[8], anaemias[9, 10], cholesterol processing[11], Waardenburg syndrome[10], Hirschsprung disease[12], glaucoma[13], cancer[14], neurological and psychatric disorders[15-17], tissue regeneration[18], congenital and acquired deafness[10, 19], neural and retinal degeneration[20, 21] as well as infectious diseases[22, 23]. In some cases, a novel disease gene was discovered first in the zebrafish and only subsequently a human disease was linked to it, such as the iron transporter ferroprotein gene in haemochromatosis type 4, a disorder in iron metabolism leading to anaemia in humans[24]. Thus, zebrafish are not only excellent models for deciphering the pathophysiology of human diseases but serve also as effective tools to discover new human disease genes (for example[25]).

In addition, small fish models are ideal for generating and experimentally validating large data-sets in vivo, making them particularly suited to the genome-wide approaches favored by systems biology[26]. By capitalizing on their transparency, the small size of the embryos, the ease of introducing markers and the possibility of achieving optical resolution at cellular and sub-cellular level, these small fishes provide vertebrate models that allow analysis of development and organ formation at a systems level in living organisms[27, 28]. Such processes can be studied against the disturbances introduced by genetic mutations and chemicals eventually allowing the integration of data into a virtual model of a vertebrate embryo.

The zebrafish is attracting increasing attention from the pharmaceutical industry as it is highly amenable to whole animal drug screening[29] and toxicological

studies[30-32]. First examples have already appeared in the literature where drug candidates were successfully identified by screening for suppression of complex genetic defects (phenotypic rescue) in zebrafish embryos[33]. In Germany, the zebrafish embryo toxicity assay has become a DIN standard for testing the water quality of sewage plants[34]. Moreover, molecular studies suggest that the zebrafish embryo may offer a suitable alternative model for systematic testing of chemicals under the European REACH initiative[35].

The rising interest in this field is encouraging an increasing number of researchers within the biomedical community to consider using small fish. However, the highly fragmented research endeavor and a limited awareness of the specific research topics pursued by each European laboratory, leaves a lot to be improved with respect to concerted scientific approaches and strategies. Moreover, the full potential of zebrafish has only started to be recognized in the industrial and regulatory sector. To overcome this, we have established the European Network on Fish Biomedical Models (EuFishBioMed) and successfully applied for sponsoring by the EU COST programme (http://www.cost.eu/domains_actions).

EuFishBioMed, the European network of zebrafish researchers

The necessity for a European network of zebrafish researcher was recognized in discussions held at a break-out session of the 5th European Zebrafish Meeting in Amsterdam, in 2007. In particular, the complex funding structure in Europe with both national and centralized European resources demanded a visible Europe-wide organisation that could lobby for funding opportunities and infrastructure for zebrafish research. From the beginning, it was clear that this network should not only include researchers working with zebrafish but also laboratories using other small fish species in their biomedically oriented research, such as medaka and swordtail. The European COST programme (http://www.cost.eu/domains_actions) with its aim of fostering scientific collaboration across Europe appeared most appropriate for funding of such an initiative. By winning support through a COST Action for the years 2009 to 2013, the European zebrafish community was endowed with the resources to firmly establish the networking infrastructure of EuFishBioMed.

One of the objectives of EuFishBioMed is to promote research on and use of

small fish as models for human diseases by providing a communication platform. To this end, a website was established at the Institute of Toxicology and Genetics (ITG) of the Karlsruhe Institute of Technology (KIT). The database of EuFishBioMed lists 353 principal investigators from 23 European countries as network members. Interested parties from outside of Europe are welcome too: in particular colleagues from Australia and New Zealand eagerly subscribed to the information network, with both countries being partners in the COST programme. In addition, we count members from India, Chile, Singapore, USA and Japan.

Lobby work to promote Europe-wide standardized protocols for fish husbandry as well as regulatory guidelines concerning experimental work with these fish models ([31] and http://eufishbiomed.kit.edu) is an on-going activity of EuFishBioMed. This is particularly important in view of the differences in national and regional regulatory guidelines in Europe. For example even within Germany, different regulatory opinions exist as to when a zebrafish is regarded as an animal and when experimentation should thus be subject to approval by the authorities. EuFishBioMed has gained an important role as competent advisor and authority in these matters across Europe[31].

EuFishBioMed has been organising workshops and meetings (Table 1) to bring together leading experts in the field and to allow young researchers and investigators to get insight into research with fish models. Topics covered by these meetings have ranged from sperm freezing, neurobiology, behavioral analysis and disease models to automation and digital image processing. To foster strategic discussions, we have established a European Zebrafish Principal Investigator Meeting (EZPM), an international meeting for principal investigators from all over the world[36]. This meeting takes place every two years alternating with the Strategic Conference of Zebrafish Investigators in Asilomar, USA. An additional important objective is the outreach to the wider biomedical research community, to industry and regulators to facilitate translation of zebrafish research into medical and industrial applications. To this end, workshops on disease models or specific applications such as toxicology have been held with participants from interested parties outside the zebrafish field (Table 1).

Lectures are well suited for information exchange but fail to teach manual skills. One of the main aims of EuFishBioMed was to enhance the transfer of technology between zebrafish research groups and make experimental protocols available to groups new to fish research. EuFishBioMed therefore also co-sponsors training schools and funds short term scientific missions (i.e. visits of graduate students and postdocs) to laboratories in Europe to learn zebrafish techniques with relevance for the research in the home laboratory. This offer is specifically available for young researchers. So far, the COST Action EuFishBioMed has funded 27 travel grants. This instrument has turned out to be a most effective motor for research collaborations across Europe and at the same time efficiently promotes the scientific training of early stage researchers.

Establishment of a European Zebrafish Resource Center

An additional important aim of EuFishBioMed is to lobby for national and European funding and to develop the necessary infrastructure for the use of fish models in biomedical research in Europe. Several white papers were written and submitted to national as well as European funding bodies (http://eufishbiomed.kit.edu/eufishbiomed/eufish_downloads.htm). These papers highlight research areas to which zebrafish can specifically contribute as a model.

A specific deficit in Europe has been the lack of a centralized infrastructure to maintain and distribute zebrafish lines and mutants. In recent years, approximately 2,500 mutant and transgenic lines were generated by European research groups, mostly in large mutagenesis screens organised as part of the ZF-MODELS project (http://zf-health.org/zf-models/information/contact.html). The potential of these fish lines including their possible use by the biotechnology industry is far from being exploited. Most of these lines are still kept in the labs in which they were created. The only option to assure their long-term maintenance is to transfer them to the Zebrafish International Resource Center (ZIRC) in the USA. Due to the difficulty of international shipping, future access of European researchers to these lines will be severely impaired. To prevent this loss for European research, EuFishBioMed lobbied successfully for support to build a European Zebrafish Resource Center (EZRC). This unique European infrastructure located at the Karlsruhe Institute of Technology, Karlsruhe, Germany has recently become operational and will closely collaborate

with ZIRC. In addition to maintaining and distributing zebrafish stocks, it will provide access to critical technology platforms (automated screening, high-throughput imaging, data storage, mapping and expression profiling). Thus, EZRC and ZIRC will complement each other in their services to the international community.

The Future: The European Society for Fish Models in Biology and Medicine

Although the administrative burden associated with a COST Action is enormous in relation to the allocated funds, there is general agreement among the scientists concerned that the EuFishBioMed COST Action (BM0804) has had a major impact on European research in this field. The establishment of a well-connected European zebrafish community with a common communication platform and resource centre has been critical to meet the specific needs of zebrafish researchers and their institutions. EuFishBioMed has become a platform for the realisation of new research projects dedicated to more specific clinical, industrial or regulatory needs. Most importantly, it has provided the tools to efficiently lobby for support without unnecessarily duplicating efforts in the complex European funding and regulatory landscape.

EU COST Action funding will cease in 2013, raising the question of how to continue afterwards. To this end, at the occasion of the 2011 European Zebrafish Meeting in Edinburgh the EuFishBioMed management committee made a decision to found a charitable association. This association will be named the European Society for Fish Models in Biology and Medicine. It will not only maintain the acronym EuFishBioMed but hopefully also continue and expand the key services currently offered by EuFishBioMed, including its website, database and the organisation and sponsoring of workshops and meetings.

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Table 1 Workshops and meetings supported by EuFishBioMed

Title	Location	Date
6th European Zebrafish Genetics and Development Meeting,	Rome, Italy	15.-19.07.2009
Disease Modelling in Zebrafish: Cancer and Immune Responses	Spoleto, Italy	20.-22.07.2009
1st European Zebrafish PI Meeting	Padua, Italy	18.-21.03.2010
In-vitro fertilization and cryo-preservation of sperm in medaka fish	Karlsruhe, Germany	29.-31.03.2010
The zebrafish embryo model in toxicology and teratology	Karlsruhe, Germany	02.-03.09.2010
Zebrafish as a neurophysiological and neurobehavioural model	London, United Kingdom	08.-09.09.2010
BioImage Analysis Workshop	Karlsruhe, Germany	01.10.2010
"Cutting Edge Technologies in Biomedical Research"	Karlsruhe, Germany	04.-06.05.2011
7th European Zebrafish Development and Genetics Meeting	Edinburgh, United Kingdom	05.-09.07.2011
Blood, Immunity, Cancer and Endothelium workshop "Zebrafish Disease Models (ZDM4)"	Edinburgh, United Kingdom	09.-11.07.2011
"Zebrafish: an animal model in biomedical research"	Utrecht, The Netherlands	14.-15.11.2011

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